

# Risk Factors for Hepatitis B Virus Infection Among Heterosexuals Attending a Sexually Transmitted Diseases Clinic in Italy: Role of Genital Ulcerative Diseases

Rosamaria Corona, Federico Caprilli, Amalia Giglio, Tommaso Stroffolini, Maria Elena Tosti, Giulio Gentili, Grazia Prignano, Paolo Pasquini, and Alfonso Mele

*Servizio di Epidemiologia, Istituto Dermopatico dell'Immacolata (IDI-IRCCS) (R.C.), Istituto Ospedaliero Dermosifilopatico di S. Maria e S. Gallicano (F.C., A.G., G.G., G.P.), Laboratorio di Epidemiologia e Biostatistica, Istituto Superiore di Sanità (M.E.T., P.P., A.M.), Ospedale di Montefiascone, Viterbo (T.S.), Italy*

The seroprevalence and risk factors for hepatitis B virus (HBV) infection were determined among 1,497 heterosexuals with no history of intravenous drug use (median age 32, range 15–78) treated at a sexually transmitted disease clinic in Rome. A total of 329 (22.0%) had antibodies to HBV core antigen (anti-HBc), a rate nearly four times as high as the 5.8% found recently in a national sample of young male adults, ages 18–26 years. Multiple logistic regression analysis showed that the risk of anti-HBc positivity was independently associated with male sex, increasing age, lower level of education, three or more sexual partners in the previous year, and positive syphilis serology. Lack of condom use, history of nonulcerative sexually transmitted diseases, and genital herpes were not associated with anti-HBc positivity. These findings confirm that heterosexual exposure plays an important role in the spread of HBV infection. Furthermore, these data indicate that individuals with more than two sexual partners a year and those with positive syphilis serology should be immunised against HBV. © 1996 Wiley-Liss, Inc.

**KEY WORDS:** hepatitis B, heterosexuals, genital ulcer disease

## INTRODUCTION

Sexual transmission of hepatitis B virus (HBV), primarily among homosexual men, has been well documented [Heathcote and Sherlock, 1973; Schreeder et al., 1982; Kingsley et al., 1990]. Heterosexual activity as a risk factor for HBV infection is being reported with increasing frequency. Exposure to several sexual partners and history of sexually transmitted diseases (STD) have been found to be associated with a higher preva-

lence of HBV markers [Alter et al., 1986, 1989; Rosenblum et al., 1990; Struve et al., 1990; Kvinesdal et al., 1993; Corona et al., 1991]. Genital ulcer diseases have been shown to be independent risk factors for HBV infection among both homosexuals [Osmond et al., 1993; Thomas et al., 1994] and heterosexuals [Rosenblum et al., 1990; Thomas et al., 1994].

In Italy, a dramatic decline in the incidence of HBV infection has been recently reported, along with a shift over time of the age-specific incidence rates [Stroffolini et al., 1991; D'Amelio et al., 1992]. The peak incidence rate, previously observed in children as a result of household transmission, has changed in recent years to the 15–24 age group and seems to reflect both intravenous drug abuse and sexual transmission. As sexual exposure is very common in the general population, its role as transmission route for HBV is of increasing importance in Italy.

This study evaluated the cofactors associated with HBV infection among heterosexual nonintravenous drug users treated at a clinic for sexually transmitted diseases in Rome, particularly focusing on the role played by genital ulcers or erosions. The prevalence of HBV markers in two different study periods were also compared.

## MATERIALS AND METHODS

### Patient Population

A study of HIV seroprevalence was conducted at the STD outpatient clinic, S. Maria e S. Gallicano Hospital in Rome, from February to December 1989 and from January 1993 to April 1994. As part of this study, an investigation of the risk factors of hepatitis B infection

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Address reprint requests to Dr. Rosamaria Corona, Servizio di Epidemiologia, Istituto Dermopatico dell'Immacolata (IDI-IRCCS), Via Monti di Creta 104, 00167 Rome, Italy.

This work was performed at the S. Maria e S. Gallicano Hospital, Rome, Italy.

among heterosexual STD patients was undertaken. Homo/bisexuals, intravenous drug users, and patients who had received blood transfusions were excluded from the study.

During the first period, all the patients seen at the STD clinic were enrolled in the study; in the second period, all patients reporting genital ulcer or erosion and in a random day a week, all patients attending the clinic were recruited. After informed consent, all patients provided a blood sample and an anonymous questionnaire was administered by a trained interviewer eliciting basic socio-demographic information (age, sex, education), sexual preference, number of sexual partners, and use of condoms in the previous year, history of intravenous drug use, blood transfusions, and STD.

Patients were considered as having genital ulcer if they had at the time of the visit to the clinic one of the following conditions: (1) a diagnosis of primary syphilis, (2) a diagnosis of secondary syphilis, (3) a diagnosis of latent syphilis, i.e., a reactive syphilis serology without apparent signs of syphilis, (4) a current diagnosis of genital herpes, or (5) a past history of genital herpes. Nonulcerative STDs included gonorrhoea, genital warts, nongonococcal urethritis (NGU) due to *Chlamydia trachomatis* or *Ureaplasma urealyticum*, vaginal or cervical infection due to *Trichomonas vaginalis*, *Candida albicans* or *Chlamydia*, scabies, and *Phthirus pubis* infestation.

### Laboratory Methods

Antibodies against hepatitis B core antigen (anti-HBc) were determined using a commercially available ELISA (Abbott Laboratories, North Chicago, IL). Syphilis serological testing was carried out with the rapid plasma reagin (RPR) test for screening and the haemagglutination test for *Treponema pallidum* (TPHA) and an EIA test for IgM and IgG for confirmation.

### Statistical Analysis

Chi-square was used to test differences between proportions where appropriate. A *P* value < 0.05 was considered to be significant. The crude odds ratios (OR) were calculated for each considered risk factor by univariate analysis. A logistic regression model [BMDP, 1990] was used to identify variables independently associated with anti-HBc positivity. Subjects reporting a past history of genital herpes and subjects with current disease were included in the model as a single category; likewise subjects with any stage syphilis were grouped for the purpose of multivariate analysis. For each variable the reference category was the most favourable level of exposure (youngest age, female sex, highest education level, lowest number of sexual partners, use of condoms, negative history of STDs).

## RESULTS

A population group of 1,770 patients (1090 in 1989, 680 in 1993–94) were asked to participate in the study. A total of 975 patients in the first period and 522 patients

TABLE I. General Characteristics of Two Groups of Heterosexual Nonintravenous Drug Users Attending a Clinic for Sexually Transmitted Diseases in Rome, Italy

	Study year			
	1989		1994	
	No. total = 975		No. total = 552	
	No.	(%)	No.	(%)
Sex				
female	296	(30)	154	(30)
male	679	(70)	368	(70)
Age				
15–24	200	(20)	105	(20)
25–34	358	(37)	195	(37)
≥35	417	(43)	222	(43)
Years of education				
>8	451	(47)	307	(59)
≤8	516	(53)	215	(41)
No. partners in previous year				
0–2	667	(69)	371	(71)
≥3	307	(31)	150	(29)
Use of condom in previous year				
Yes	187	(20)	148	(28)
No	743	(80)	374	(72)
No history of STD	450	(46)	252	(50)
History of nonulcerative STD	310	(32)	105	(21)
Current genital herpes	43	(4)	90	(18)
Past history of genital herpes	16	(2)	7	(1)
Primary syphilis	5	(0.5)	2	(0.4)
Secondary syphilis	5	(0.5)	4	(1)
Latent syphilis	146	(15)	41	(8)

in the second period provided a blood sample and were enrolled. The acceptance rates were 89.4% and 76.8%, respectively. Out of 1,497 patients, 1,047 were male; the median age was 32 years (range 15–78).

The general characteristics of the two groups of patients included in the study are shown in Table I. The two groups were similar regarding sex, age, number of sexual partners in the previous year, and history of STD. The proportion of patients with a higher level of education was slightly greater in the 1993–94 group as compared with the 1989 group. Patients seen in 1993–94 also were more likely to have used condoms in the previous year than patients seen in 1989. Out of 156 patients with genital herpes, 133 (85%) had current disease at the time of the enrollment into the study, and 23 (15%) reported a history of genital herpes. Of the 203 patients with positive syphilis serology, 16 (8%) had primary or secondary syphilis and 187 (92%) had latent syphilis.

The overall anti-HBc prevalence in the study population was 22%; however, the anti-HBc positivity rate decreased by 27% from 1989 to 1993–94 (24.3% vs. 17.6%, *P* < 0.01). The decline was observed either among patients without history of STDs (20.1% vs. 13.5%, *P* < 0.03) or those with genital ulcer diseases (38.6% vs. 26.4%, *P* < 0.01).

The crude OR linking anti-HBc positivity with risk factors of interest, estimated by univariate analysis, are shown in Table II: an association with anti-HBc positivity was found for male sex, increasing age, lower education level, three or more sexual partners in the previous

TABLE II. Risk Factors Associated With Anti-HBc Positivity Among 1,497 Heterosexual Nonintravenous Drug Users Attending a Sexually Transmitted Diseases Clinic in Rome

Characteristics	No. positive/ No. tested	(%)	O.R. <sup>a</sup>	95% C.I. <sup>b</sup>
Sex				
female	64/450	(14.2)	1	
male	265/1047	(25.3)	2.04	(1.52–2.76)
Age				
15–24	19/305	(6.2)	1	
25–34	86/553	(15.6)	2.77	(1.65–4.66)
≥35	224/639	(35.1)	8.12	(4.97–13.3)
Years of education				
>8	104/758	(13.7)	1	
≤8	223/731	(30.5)	2.76	(2.12–3.58)
No. partners in previous year				
0–2	205/1038	(19.7)	1	
≥3	124/457	(27.1)	1.51	(1.17–1.96)
Use of condom in previous year				
Yes	71/335	(21.2)	1	
No	243/1117	(21.8)	1.03	(0.76–1.39)
No history of STD	127/702	(18.1)	1	
History of nonulcerative STD	78/415	(18.8)	1.05	(0.76–1.45)
Current genital herpes	28/133	(21.1)	1.21	(0.74–1.95)
Past history of genital herpes	2/23	(8.7)	0.43	(0.05–1.80)
Primary or secondary syphilis	4/16	(25.0)	1.51	(0.35–5.08)
Latent syphilis	87/187	(46.5)	3.94	(2.75–5.65)

<sup>a</sup>Crude Odds Ratios (O.R.) derived from univariate analysis.<sup>b</sup>Confidence interval.

year, and positive syphilis serology. Failure to use condoms in the previous year, history of nonulcerative STDs, and current or past genital herpes were not associated with anti-HBc positivity.

Adjustment of each variable for the confounding effect of the other variables was done by logistic regression. No association was found for patients reporting nonulcerative STDs or genital herpes, whereas male sex, increasing age, lower education level, three or more sexual partners in the previous year, and positive syphilis serology were all independent risk factors for HBV infection (Table III). In particular, patients with a positive test for syphilis had a 2.4-fold risk of anti-HBc positivity (95% confidence interval, 1.64–3.40) when compared with those without STD history.

## DISCUSSION

The present study confirms that heterosexual exposure plays an important role in the spread of HBV infection. The overall prevalence of anti-HBc positivity among heterosexual patients treated at STD clinic in Rome in 1989 and in 1993–1994 was 22%, the median age for the two groups was 32 years, and these individuals denied intravenous drug use and homosexuality. This rate is nearly four times as high as 5.8% found in a recent study on a national sample of young adult males between 18–26 years of age [D'Amelio et al., 1992]. However, a significant decrease of anti-HBc prevalence was observed from 1989 to 1993–1994, reflecting the general decline of HBV endemicity in the Italian population in recent years [Stroffolini et al., 1991; D'Amelio et al., 1992]. The prevention campaign against the HIV infection also may have played a role, as suggested by the

TABLE III. Variables Associated With Anti-HBc Positivity Among 1,497 Heterosexual Nonintravenous Drug Users Attending a Sexually Transmitted Diseases Clinic in Rome

Risk factors	O.R. <sup>a</sup>	95% C.I. <sup>b</sup>
Sex		
female	1	
male	1.85	(1.32–2.59)
Age		
15–24	1	
25–34	2.75	(1.62–4.69)
≥35	6.64	(4.00–11.0)
Years of education		
>8	1	
≤8	2.35	(1.77–3.12)
No. partners in previous year		
0–2	1	
≥3	1.43	(1.06–1.91)
No history of STD	1	
History of nonulcerative STD	0.97	(0.69–1.35)
Genital herpes	0.83	(0.52–1.33)
Positive syphilis serology	2.36	(1.64–3.40)

<sup>a</sup>Adjusted O.R. (odds ratio) derived from multiple logistic regression model. Each variable is adjusted for all the other variables in the table.<sup>b</sup>Confidence interval.

increasing proportion over time of subjects reporting the use of condoms (20% in 1989, 28% in 1993–1994,  $P < 0.005$ ).

Multivariate analysis of risk factors showed that male sex, increasing age, lower education level, more than two sexual partners, and reactive syphilis serology were all independently associated with the risk of HBV infection. A lower level of education is a good indicator of low socio-economic status, which is not a risk factor in

itself but may identify lifestyles, behaviours, or habits that increase the probability of HBV infection.

This study provides clear evidence that subjects with either primary, secondary, or latent syphilis are at increased risk of HBV infection. These findings confirm the results of a large study on seroprevalence and risk factors of HBV infection in the United States, which showed that a positive antibody test for syphilis is strongly associated with HBV [McQuillan et al., 1989]. Syphilis may be a risk factor for HBV infection for several reasons. First, it can be considered an indicator of contact with individuals at increased risk for HBV infection. Second, it may be a cofactor in sexual transmission of HBV, as the disruption of the epithelial surface resulting in a genital ulcer may increase the efficiency of HBV transmission. The lack of mucosal integrity may allow direct inoculation of HBV, whose presence has been demonstrated in genital secretions [Heathcote et al., 1974; Darani and Gerber, 1974]. Accordingly, epithelial disruptions easily produced by trauma associated with anogenital intercourse explain the HBV rate generally higher in homosexual than in heterosexual men [Dietzman et al., 1977; Mele et al., 1988].

In the present study, an association with present or past history of genital herpes, which also causes genital ulceration, was not found. These results are consistent with those of Thomas et al. [1994]. It is speculated that biological factors might affect the role of the disease as cofactor in HBV transmission (e.g., the painful herpetic lesion could deter from sexual activity). Alternatively, genital herpes could merely be a worse surrogate marker of risk sexual behaviour than syphilis.

Behavioural factors, such as multiple sexual partners, have been shown to increase sexual transmission of HBV [Alter et al., 1986, 1989; Rosenblum et al., 1990]. In our study, subjects reporting more than two sexual partners in the previous year were more likely to present anti-HBc (O.R. 1.41; 95% C.I. 1.05–1.90). However, in a recent study conducted among heterosexuals attending a STD clinic in Amsterdam, no association between HBV infection and number of different sexual partners was found [Van Doormun et al., 1994]. An explanation for this discrepancy may be that in the Dutch study only subjects with five or more sexual partners in the previous 6 months were included; thus all participants reported a high number of sexual partners.

HBV infection and condom use were not associated in our study. Information on condom use was collected only for the year previous to the clinic visit; hence the data may not reflect past unprotected sexual activity when HBV infection may have been acquired.

In conclusion, the results of this study provide clear indications for an HBV immunisation policy. Heterosexuals who have more than two sexual partners in a year and those reporting sexually transmitted diseases with genital ulceration should be immunised for HBV. Data from national surveillance of acute viral hepatitis suggest that more than one-third of young and adult cases of acute B hepatitis report exposure to multiple sexual partners [Mele et al., 1995]. The control of the above

risk factor will contribute to the further decline of HBV infection in Italy.

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